

WHAT IS CLAIMED IS:

1. A method of obtaining a centromere nucleic acid sequence from a selected organism comprising the steps of
 - a) preparing a first sample of genomic DNA from a selected organism;
 - 5 b) obtaining a plurality of methylated nucleic acid segments from said genomic DNA; and
 - c) screening said methylated nucleic acid segments to identify a centromere nucleic acid sequence.
- 10 2. The method of claim 1, wherein said obtaining comprises contacting said genomic DNA with a methylation sensitive restriction endonuclease and selecting nucleic acid segments exhibiting resistance to cleavage with said methylation sensitive restriction endonuclease to obtain said plurality of methylated nucleic acid segments.
- 15 3. The method of claim 1, wherein the plurality of methylated nucleic acid segments is further defined as comprising hemimethylated nucleic acid segments.
4. The method of claim 1, wherein said obtaining comprises immunoprecipitating said methylated nucleic acid segments with an antibody capable of specifically binding
20 methylated DNA.
5. The method of claim 4, wherein said obtaining comprises immunoprecipitating said methylated nucleic acid segments with an antibody capable of specifically binding protein associated with the methylated nucleic acid segments.
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6. The method of claim 1, further defined as comprising labeling at least a first methylated nucleic segment from said plurality of methylated nucleic acid segments, hybridizing said first methylated nucleic segment to a clone comprising genomic DNA of said selected organism and detecting said labeling to obtain a clone comprising a centromere nucleic
30 acid sequence.

7. The method of claim 1, wherein said screening comprises the steps of
- a) obtaining an array comprising cloned genomic DNA from said selected organism;
 - b) detecting a candidate centromere nucleic acid sequence from said cloned genomic DNA of said array, said candidate centromere nucleic acid sequence comprising a nucleic acid sequence complementary to a nucleic acid sequence of at least a first member of said plurality of methylated nucleic acid segments; and
 - c) identifying a centromere nucleic acid sequence from said candidate centromere sequence.
8. The method of claim 7, wherein said detecting is further defined as comprising detecting a plurality of candidate centromere nucleic acid sequences from said array, said candidate centromere nucleic acid sequences comprising nucleic acid sequences complementary to a nucleic acid sequence of at least a first member of said plurality of methylated nucleic acid segments.
9. The method of claim 7, wherein said array comprises said cloned genomic DNA attached to a solid support.
10. The method of claim 9, wherein said array is further defined as comprising cloned genomic DNA attached to said solid support in a selected pattern
11. The method of claim 10, wherein said selected pattern comprises a grid.
12. The method of claim 9, wherein said cloned genomic DNA comprises DNA cloned in a bacterial artificial chromosome.
13. The method of claim 9, wherein said cloned genomic DNA comprises DNA cloned in a yeast artificial chromosome.
14. The method of claim 9, wherein the solid support comprises a microscope slide.

15. The method of claim 7, wherein said detecting comprises fluorescently labeling said plurality of methylated nucleic acid segments and hybridizing the labeled plurality of methylated nucleic acid segments to said array.

5 16. The method of claim 7, wherein said detecting comprises labeling said plurality of methylated nucleic acid segments with an antigen, hybridizing the labeled plurality of methylated nucleic acid segments to said array and detecting said antigen with a molecule which binds said antigen.

10 17. The method of claim 9, wherein said solid support comprises a hybridization filter.

18. The method of claim 7, wherein said detecting comprises radioactively labeling said plurality of methylated nucleic acid segments and hybridizing the labeled plurality of methylated nucleic acid segments to said array.

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19. The method of claim 7, wherein said array comprises a plurality of DNA pools, said pools comprising the nucleic acid sequences of at least a first and a second clone comprising genomic DNA from said selected organism.

20 20. The method of claim 2, wherein said contacting is further defined as comprising:

- a) obtaining a second sample of genomic DNA from said selected organism;
- b) contacting said second sample of genomic DNA with an isoschizomer of said methylation sensitive restriction endonuclease, wherein said isoschizomer is not methylation sensitive;

25 c) resolving separately said first and said second samples of genomic DNA following said contacting with said isoschizomer and said methylation sensitive restriction endonuclease; and

d) selecting a plurality of methylated nucleic acid segments from at least a first nucleic acid fraction present in said first sample of genomic DNA and not present
30 in said second sample of genomic DNA.

21. The method of claim 20, further defined as comprising contacting said second sample of genomic DNA with said methylation sensitive restriction endonuclease.

22. The method of claim 1, wherein said methylation sensitive restriction endonuclease is selected from the group consisting of: AatII, AccIII, Acil, AfaI, AgeI, AhaII, Alw26I, Alw44I, ApaLI, ApyI, AscI, Asp718I, AvaI, AvaII, Bme216I, BsaAI, BsaHI, BscFI, BsiMI, BsmAI, BsiEI, BsiWI, BsoFI, Bsp105I, Bsp119I, BspDI, BspEI, BspHI, BspKT6I, BspMII, BspRI, BspT104I, BsrFI, BssHII, BstBI, BstEIII, BstUI, BsuFI, BsuRI, CaeI, CboI, CbrI, CceI, Cfr10I, ClaI, Csp68KII, Csp45I, CtlI, CviAI, CviSIII, DpnII, EagI, Ecl136II, Eco47I, Eco47III, EcoRII, EcoT22I, EheI, Esp3I, Fnu4HI, FseI, FspI, Fsp4HI, GsaI, HaeII, HaeIII, HgaI, HhaI, HinPII, HpaII, HpyAIII, ItaI, KasI, Kpn2I, LlaAI, LlaKR2I, MboI, MflI, MluI, MmeII, MroI, MspI, MstII, MthTI, NaeI, NarI, NciAI, NdeII, NgoMIV, NgoPII, NgoS II, NlaIII, NlaIV, NotI, NruI, NspV, PmeI, PmlI, Psp1406I, PvuI, RalF40I, RsaI, RspXI, RsrII, SacII, SalI, Sau3AI, SexAI, SfoI, SfuI, SmaI, SnaBI, SolI, SpoI, SspRFI, Sth368I, TaiI, TaqI, TfiI, TthHB8I, VpaK11BI, XhoI

23. The method of claim 20, wherein said isoschizomer is selected from the group consisting of: AccIII, AflI, Alw26I, Alw44I, AmaI, AorI, ApaLI, ApyI, AspMDI, BamFI, BamHI, BamKI, BanII, BbeI, BbsI, Bce243I, Bfi57I, BpmI, BsaBC3I, BsaHI, BsaJI, BsaWI, BshGI, BsiLI, BsmI, BsmAI, BsoBI, BsoFI, Bsp122I, Bsp1286I, Bsp143I, Bsp143II, Bsp2095I, Bsp49I, Bsp51I, Bsp52I, Bsp54I, Bsp56I, Bsp57I, Bsp58I, Bsp59I, Bsp60I, Bsp61I, Bsp64I, Bsp65I, Bsp66I, Bsp67I, Bsp72I, Bsp91I, BspAI, BspEI, BspFI, BspJ64I, BspLI, BspMI, BspMII, BsrBI, BsrPII, BstI, Bst2UI, BstEII, BstNI, BstOI, BstYI, Bsu36I, BtcI, BuaI, CbiI, CceI, CcyI, CpfI, Csp5I, Csp6I, CviAII, CviQI, Eam1105I, EarI, EcoOI09I, EcoRI, EcoRV, EheI, EsaBC4I, FnuEI, FokI, HaeIII, HgiAI, HpaII, HphI, ItaI, KasI, KpnI, Kpn2I, Kzo9I, Mabl, MboI, MroI, MspI, MspBI, MssI, MvaI, NarI, NdeII, NgoPII, NsiI, PaeR7I, PagI, Pei9403I, PfaI, PmeI, PspGI, PsuI, SacI, SalDI, Sau3AI, SauMI, Sbo13I, SfaNI, SfuI, SphI, Sth368I, TaqI, TaqXI, TfiI, Tth111I, XhoII, XmaI, ZaiI

24. The method of claim 1, wherein the resistance to cleavage with said methylation sensitive restriction endonuclease is determined by a method comprising measuring the length of said methylated nucleic acid segments following said contacting.
- 5 25. The method of claim 24, wherein the average length of said plurality of methylated nucleic acid segments is at least 3 kb.
26. The method of claim 24, wherein the average length of said plurality of methylated nucleic acid segments is at least 5 kb.
- 10 27. The method of claim 24, wherein the average length of said plurality of methylated nucleic acid segments is at least 10 kb.
- 15 28. The method of claim 1, further defined as comprising obtaining a plurality of unmethylated nucleic acid segments and comparing said plurality of unmethylated nucleic acid segments to said plurality of methylated nucleic acid segments to identify at least a first methylated nucleic acid segment present in the plurality of methylated nucleic acid segments and not present in the plurality of unmethylated nucleic acid segments.
- 20 29. The method of claim 7, further defined as comprising hybridizing a plurality of unmethylated nucleic acid segments to one or both of said first methylated nucleic acid segment or said clone comprising genomic DNA of said selected organism, wherein said plurality of unmethylated nucleic acid segments have not received said labeling.
- 25 30. The method of claim 28, wherein said obtaining a plurality of unmethylated nucleic acid segments comprises identifying a plurality of nucleic acid sequences which are susceptible to restriction with said methylation sensitive restriction endonuclease.
- 30 31. The method of claim 30, further defined as measuring an average length of said plurality of unmethylated nucleic acid segments following restriction with said methylation sensitive restriction endonuclease.

32. The method of claim 31, wherein said average length of said plurality of unmethylated nucleic acid segments is less than 5 kb following restriction with methylation sensitive restriction endonuclease.
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33. The method of claim 31, wherein said average length of said plurality of unmethylated nucleic acid segments is less than 3 kb following restriction with said methylation sensitive restriction endonuclease.
- 10 34. The method of claim 1, wherein said selected organism is a plant.
35. The method of claim 34, wherein said plant is a dicotyledonous plant.
- 15 36. The method of claim 35, wherein said dicotyledonous plant is selected from the group consisting of tobacco, tomato, potato, sugar beet, pea, carrot, cauliflower, broccoli, soybean, canola, sunflower, alfalfa, cotton and *Arabidopsis*.
37. The method of claim 36, wherein said dicotyledonous plant is *Arabidopsis thaliana*.
- 20 38. The method of claim 34, wherein said plant is a monocotyledonous plant.
39. The method of claim 38, wherein said monocotyledonous plant is selected from the group consisting of wheat, maize, rye, rice, turfgrass, oat, barley, sorghum, millet, and sugarcane.
- 25 40. The method of claim 39, wherein said monocotyledonous plant is maize.
41. The method of claim 1, wherein said selected organism is a mammal.
- 30 42. The method of claim 1, wherein said selected organism is a human.

43. The method of claim 7, wherein said screening comprises identifying a candidate centromere sequence not comprising repetitive DNA.
44. The method of claim 1, wherein said contacting comprises:
- 5 a) incubating said genomic DNA with said methylation sensitive restriction endonuclease to digest unmethylated DNA;
- b) resolving digested genomic DNA from undigested genomic DNA by electrophoresis; and
- 10 c) isolating said plurality of methylated nucleic acid segments away from the undigested genomic DNA.
45. The method of claim 44, wherein the average length of said plurality of methylated nucleic acid segments is at least 3 kb.
- 15 46. The method of claim 44, wherein the average length of said plurality of methylated nucleic acid segments is at least 5 kb.
47. The method of claim 44, wherein the average length of said plurality of methylated nucleic acid segments is at least 10 kb in length.
- 20 48. The method of claim 1, further defined as comprising fluorescent *in situ* hybridization of at least a first methylated nucleic acid segment from said plurality of methylated nucleic acid segments.
- 25 49. The method of claim 1, further defined as comprising determining the nucleic acid sequence of at least a first methylated nucleic acid segment from said plurality of methylated nucleic acid segments.
- 30 50. The method of claim 49, further defined as comprising comparing the nucleic acid sequence of said first methylated nucleic acid segment to a known centromere sequence.

51. The method of claim 49, further defined as comprising immunoprecipitating centromere nucleic acid sequence and comparing said sequence to the nucleic acid sequence of said first methylated nucleic acid segment.
- 5 52. The method of claim 51, further defined as comprising immunoprecipitating said centromere nucleic acid sequences with an antibody capable of binding methylated DNA.
53. The method of claim 51, further defined as comprising immunoprecipitating said centromere nucleic acid sequences with an antibody capable of binding a centromere-associated protein.
- 10 54. The method of claim 1, further defined as comprising genetically mapping at least a first methylated nucleic acid segment from said plurality of methylated nucleic acid segments.
- 15 55. The method of claim 1, further defined as comprising determining the extent of acetylation of at least a first histone bound to at least a first methylated nucleic acid segment from said plurality of methylated nucleic acid segments.
- 20 56. The method of claim 1, further defined as comprising transforming a cell with at least a first methylated nucleic acid segment from said plurality of methylated nucleic acid segments.
57. The method of claim 56, wherein said cell is transformed with said methylated nucleic acid segment.
- 25 58. The method of claim 57, wherein said cell is further defined as integratively transformed with said methylated nucleic acid segment.
- 30 59. The method of claim 57, wherein said cell is further defined as non-integratively transformed with said methylated nucleic acid segment.

60. The method of claim 58, wherein said screening comprises observing a phenotypic effect present in the integratively transformed cells or an organism comprising the cells, wherein said phenotypic effect is absent in a control cell not integratively transformed with said methylated nucleic acid segment, or a n organism comprising said control cell.

61. The method of claim 60, wherein said phenotypic effect is selected from the group consisting of reduced viability, reduced efficiency of said transforming, genetic instability in the integratively transformed nucleic acid, aberrant tissue sectors, increased ploidy, aneuploidy, and increased integrative transformation in distal or centromeric chromosome regions.

62. The method of claim 56, wherein said first methylated nucleic acid segment is further defined as comprising a recombinant construct.

63. The method of claim 56, wherein said methylated nucleic acid segment is further defined as comprising cloned DNA.

64. The method of claim 63, wherein the cloned DNA is not methylated.

65. The method of claim 63, wherein the cloned DNA is remethylated prior to said transforming.

66. The method of claim 56, wherein the methylated nucleic acid segment is hemimethylated.

67. The method of claim 62, wherein said recombinant construct comprises a telomere.

68. The method of claim 62, wherein said recombinant construct comprises an autonomous replicating sequence (ARS).

69. The method of claim 62, wherein said recombinant construct comprises a structural gene.

70. The method of claim 69, wherein said structural gene comprises a selectable or screenable marker gene.
- 5 71. A centromere nucleic acid sequence prepared by the method of claim 1.
72. A non-human organism prepared by the method of claim 56.
73. A progeny of any generation of the organism of claim 72, said organism comprising said
10 first methylated nucleic acid segment.
74. A method of obtaining a centromere nucleic acid sequence from a selected organism comprising the steps of:
- 15 a) preparing a first sample of genomic DNA from a selected organism;
- b) contacting said genomic DNA with a strand-specific methylation sensitive restriction endonuclease;
- c) nick-translating the genomic DNA; and
- c) detecting a centromere nucleic acid sequence that hybridizes to the nick-translated genomic DNA.
- 20 75. The method of claim 74, wherein the strand-specific methylation sensitive restriction endonuclease is selected from the group consisting of HpaI, KpnI, MaeII, or Sau3A I.
76. The method of claim 74, wherein detecting comprises screening an array.
- 25 77. The method of claim 76, wherein said screening comprises the steps of
- a) obtaining an array comprising cloned genomic DNA from said selected organism; and
- b) detecting a centromere nucleic acid sequence from said cloned genomic DNA of
30 said array by hybridizing the nick translated genomic DNA to said array.

78. The method of claim 77, wherein a plurality of centromere nucleic acid sequences are detected from said array.
79. The method of claim 77, wherein said array comprises said cloned genomic DNA attached to a solid support.
80. The method of claim 79, wherein said array is further defined as comprising cloned genomic DNA attached to said solid support in a selected pattern
81. The method of claim 80, wherein said selected pattern comprises a grid.
82. The method of claim 79, wherein said cloned genomic DNA comprises DNA cloned in a bacterial artificial chromosome.
83. The method of claim 79, wherein said cloned genomic DNA comprises DNA cloned in a yeast artificial chromosome.
84. The method of claim 79, wherein the solid support comprises a microscope slide.
85. The method of claim 79, wherein said solid support comprises a hybridization filter.
86. The method of claim 77, wherein said array comprises a plurality of DNA pools, said pools comprising the nucleic acid sequences of at least a first and a second clone comprising genomic DNA from said selected organism.
87. The method of claim 74, wherein said contacting is further defined as comprising:
- a) obtaining a second sample of genomic DNA from said selected organism;
 - b) contacting said second sample of genomic DNA with an isoschizomer of said strand-specific methylation sensitive restriction endonuclease, wherein said isoschizomer is not a strand-specific methylation sensitive restriction endonuclease;

- c) resolving separately said first and said second samples of genomic DNA following said contacting; and
- d) selecting a plurality of hemimethylated nucleic acid segments from at least a first nucleic acid fraction present in said first sample of genomic DNA and not present in said second sample of genomic DNA.

88. The method of claim 74, wherein said nick-translating comprises radioactively labeling the genomic DNA.

89. The method of claim 74, wherein said nick-translating comprises labeling the genomic DNA with an antigen.

90. The method of claim 74, wherein said nick-translating comprises labeling the genomic DNA with a fluorophore.

91. The method of claim 74, wherein said selected organism is a plant.

92. The method of claim 91, wherein said plant is a dicotyledonous plant.

93. The method of claim 92, wherein said dicotyledonous plant is selected from the group consisting of tobacco, tomato, potato, sugar beet, pea, carrot, cauliflower, broccoli, soybean, canola, sunflower, alfalfa, cotton and *Arabidopsis*.

94. The method of claim 93, wherein said dicotyledonous plant is *Arabidopsis thaliana*.

95. The method of claim 91, wherein said plant is a monocotyledonous plant.

96. The method of claim 95, wherein said monocotyledonous plant is selected from the group consisting of wheat, maize, rye, rice, turfgrass, oat, barley, sorghum, millet, and sugarcane.

97. The method of claim 96, wherein said monocotyledonous plant is maize.
98. The method of claim 74, wherein said selected organism is a mammal.
- 5 99. The method of claim 74, wherein said selected organism is a human.
100. The method of claim 74, further defined as comprising fluorescent *in situ* hybridization of the centromere nucleic acid sequence.
- 10 101. The method of claim 74, further defined as comprising determining the nucleic acid sequence of the centromere nucleic acid sequence.
102. The method of claim 101, further defined as comprising comparing the nucleic acid sequence of the centromere nucleic acid sequence to a known centromere sequence.
- 15 103. The method of claim 74, further defined as comprising transforming a cell with the centromere nucleic acid sequence.
104. The method of claim 103, wherein said cell is further defined as integratively transformed with said centromere nucleic acid sequence.
- 20 105. The method of claim 103, wherein said cell is further defined as non-integratively transformed with said centromere nucleic acid sequence.
- 25 106. The method of claim 104, further comprising screening for a phenotypic effect present in the integratively transformed cells or an organism comprising the cells, wherein said phenotypic effect is absent in a control cell not integratively transformed with said centromere nucleic acid sequence or an organism comprising said control cell.
- 30 107. The method of claim 106, wherein said phenotypic effect is selected from the group consisting of reduced viability, reduced efficiency of said transforming, genetic

instability in the integratively transformed nucleic acid, aberrant tissue sectors, increased ploidy, aneuploidy, and increased integrative transformation in distal or centromeric chromosome regions.

5 108. The method of claim 103, wherein said centromere nucleic acid sequence is further defined as comprising a recombinant construct.

109. The method of claim 103, wherein said centromere nucleic acid sequence is further defined as comprising cloned DNA.

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110. The method of claim 109, wherein the cloned DNA is not methylated.

111. The method of claim 109, wherein the cloned DNA is remethylated prior to said transforming.

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112. The method of claim 111, wherein the remethylated DNA is hemimethylated.

113. The method of claim 108, wherein said recombinant construct comprises a telomere.

20 114. The method of claim 108, wherein said recombinant construct comprises an autonomous replicating sequence (ARS).

115. The method of claim 108, wherein said recombinant construct comprises a structural gene.

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116. The method of claim 115, wherein said structural gene comprises a selectable or screenable marker gene.

117. A centromere nucleic acid sequence prepared by the method of claim 74.

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118. A non-human organism prepared by the method of claim 103.

119. A progeny of any generation of the organism of claim 118, said organism comprising said first methylated nucleic acid segment.